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TITLE:
*RNASEH2A* -- a Putative "Non-Oncogene Addiction" Gene Target and Marker for Radio-sensitivity in High Risk Prostate Cancer

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

We proposed that *RNASEH2A* represents a novel type of gene, up-regulated in lethal prostate cancer to prevent catastrophic genomic instability and cell death and thereby acting to make prostate cancers resistant to treatment with radiation therapy. The major findings to date include (1) independent validation of the associate of *RNASEH2A* with tumor grade. (2) Identification and sample preparation of RNA specimens with lethal potential for analysis of RNAseH2a expression.(3)Observation that RNASH2A expression does not independently predict lethal prostate cancer.(4)Observation that RNASH2A expression does predict radio-sensitivity and response to treatment in men who underwent radical prostatectomy and subsequently had post – operative radiation. (5) Analysis of prostate cells lines for endogenous expression of RNASH2a (6) construction of plasmids that allow to over express RNAseH2a in prostate cancer cell lines to evaluate their impact on radiation sensitivity.

**15. SUBJECT TERMS**

High Risk prostate cancer, radio sensitivity, biomarker

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## Introduction

Over the last two decades there have been major advances in both the detection and treatment of localized prostate cancer. Although this has resulted in a decrease in prostate cancer specific mortality, prostate cancer still remains the second leading cause of cancer related death among men. Thus, key issues in the management of prostate cancer today include the identification of men with aggressive disease and the development and improvement of therapies to treat lethal cancers. Currently, Gleason grade is the most potent forecaster of metastatic ability and prostate cancer specific mortality. As such, we have begun to investigate the molecular features of high risk prostate cancer by correlating Gleason grade with RNA based expression patterns. Our work has identified pathways of genome stability as enriched in high grade disease and specifically *RNASEH2A,* a putative chromosomal integrity determinant as one of the most strikingly over-expressed genes in aggressive prostate cancer (over 6 fold increase in Gleason 8 vs Gleason 6 tissue, $p < 1x10^{-6}$). In this proposal, we hypothesized that *RNASEH2A* is associated with lethal, high grade disease maintaining chromosomal stability. Together this proposal implicates *RNASEH2A* as a marker and modulator of radio-resistance in prostate cancer.

## Body

We proposed to in Specific Aim 1 to demonstrate the association of *RNASEH2A* with lethal prostate cancer. To establish this correlation we will examine the expression of *RNASEH2A* RNA and protein in the prostatectomy tissue of men who either rapidly progressed to metastasis and death following local therapy or those with high grade disease and favorable clinical outcomes.

In the past year we have validated our antibody and are in the process of staining our Johns Hopkins Recurrence Tissue microarray to determine if RNAaseH2A predicts cancer recurrence. The RNA expression analysis is ongoing with samples being extracted and run.

We have accomplished much work on specific Aim 2 in the last year. We have developed constructs to evaluate the ability of *RNASEH2A* over-expression and suppression on cell proliferation and radiosensitivity in a mammalian system. We have developed 2 shRNA constructs the knock down RNASH2A quite effectively by both protein (Figure 1) and RNA expression (Figure 2). Cell proliferation is significantly suppressed with proliferation affected in a suppression correlative fashion (Figure 3) (As seen in figure 1 construct A, B and C differentially suppress RNASEH2a expression on the protein level). We have also developed an over expressing RNAH2A construct as demonstrated in Figure 4.

We have begun to test these cells sensitivity to ionizing radiation. We have tested this in LnCap cells initially and have not noted any additionally resistance to radiation in LnCap cells when RNASH2a is over expressed. (Figure 5). Because RNASH2A is moderately over-expressed to begin with in these cells we are planning to test the sensitivities in various cancerous and non-cancerous cell lines. (Figure 6)

We have subsequently begun to generate xenografts of cells lines either over – expressing or under expressing RNASH2A. We are in the process of monitoring xenograft growth and hope to begin sensitivities to ionizing radiation soon. Preliminary work suggest that over expression of RNASH2A conveys a growth advantage to xenografts while shRNA suppression of RNASEH2a expression attenuated growth. (Figure 7)

In specific aim 3 we proposed that RNAasH2a is a marker of radio-sensitivity. We have evaluated RNAseH2a expression in in the Mayo Clinic cohort who underwent adjuvant or salvage radiation.. In this subset, men with high expression of RNASEH2A were statistically more likely to experience biochemical recurrence ( $p=0.005$), metastasis ( $p = 0.002$) and death ( $p = 0.02$) On multivariate analysis incorporating PSA, Gleason score, Seminal vesicle invasion, Extraprostatic extension and surgical margin status, RNASH2A expression significantly

predicted biochemical and metastatic free survival ($p < 0.001$ for both). Thus a the subset of men who underwent post-operative radiation levels of higher levels of expression of RNASH2A does appear to be associated with poorer outcomes in a statistically significant manner. We have plans to test this observation in a TMA based format to confirm these observations
We are following up these data

**Key Research Accomplishments**

- RNASH2A expression correlates with prostate cancer grade
- RNASH2A expression predicts radio-sensitivity and response to treatment in men who underwent radical prostatectomy and subsequently had post – operative radiation.
- RNASH2A expression is increased in prostate cancer cell lines
- RNASH2A expression impact cell proliferation.
- RNASH2A expression does not appear to confirm additional radio-resistance in cell line that already over- express RNASH2A.
- RNASH2A expression levels appear to affect xenograft size.

**Reportable Outcomes**

**No reportable outcomes have come from this work thus far**

**Conclusions**

To date we have demonstrated *RNASEH2A* is associated with high grade prostate cancer but is not an independent marker of lethal disease in men undergoing radical prostatectomy. However in men who underwent radical prostatectomy and experienced a recurrence, RNASH2A expression is a independent marker of radio-resistance in prostate cancer and worse clinical outcome. RNASH2A appears to regulate cell growth. The precise role of RNASH2A in radiation sensitivity remains to be determined on a cellular level

**References**

1. Ross AE, Marchionni L, Vuica-Ross M, Cheadle C, Fan J, Berman DM, Schaeffer EM., Gene expression pathways of high grade localized prostate cancer., Prostate. 2011 Feb 25.

2. Hurley PJ, Marchionni L, Simons BW, **Ross** AE, Peskoe SB, Miller RM, Erho N, Vergara IA, Ghadessi M, Huang Z, Gurel B, Park BH, Davicioni E, Jenkins RB, Platz EA, Berman DM, **Schaeffer EM**. Secreted protein, acidic and rich in cysteine-like 1 (SPARCL1) is down regulated in aggressive prostate cancers and is prognostic for poor clinical outcome. Proc Natl Acad Sci U S A. 2012 Sep 11;109(37):14977-82. Epub 2012 Aug 27.

**Appendicies**

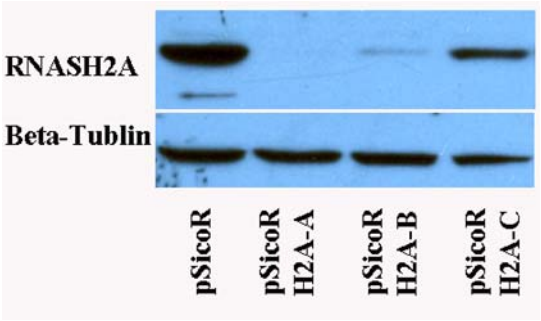


Figure 1 **Western analysis of RNASEH2A protein expression LnCap cells in the presence of shRNA constucts.**

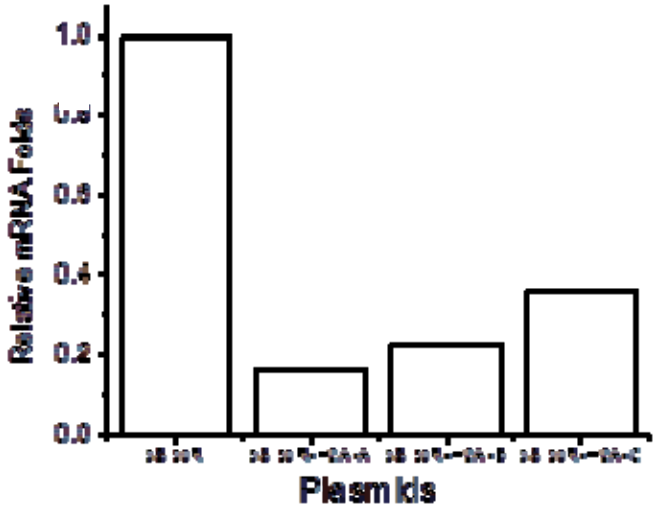


Figure 2 **RNA expression of** RNASH2A in LnCaP cells in the presence of shRNA

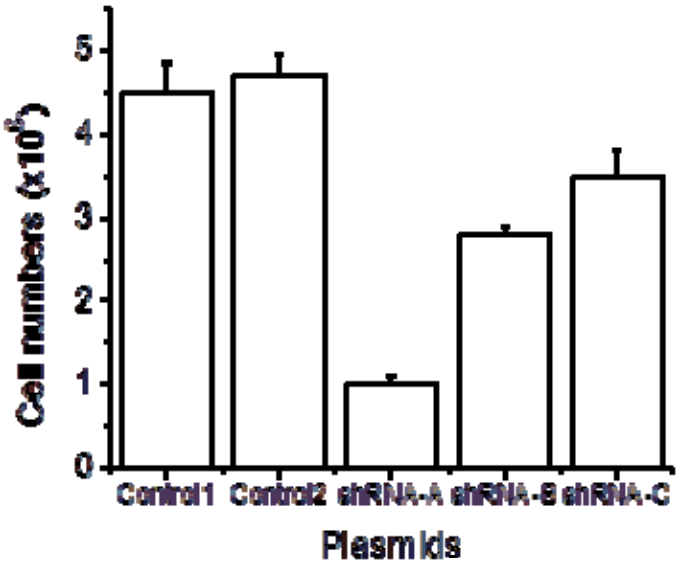


Figure 3. Proliferation of LnCaP cells in the presence of 3 shRNA constructs against RNASEH2A.

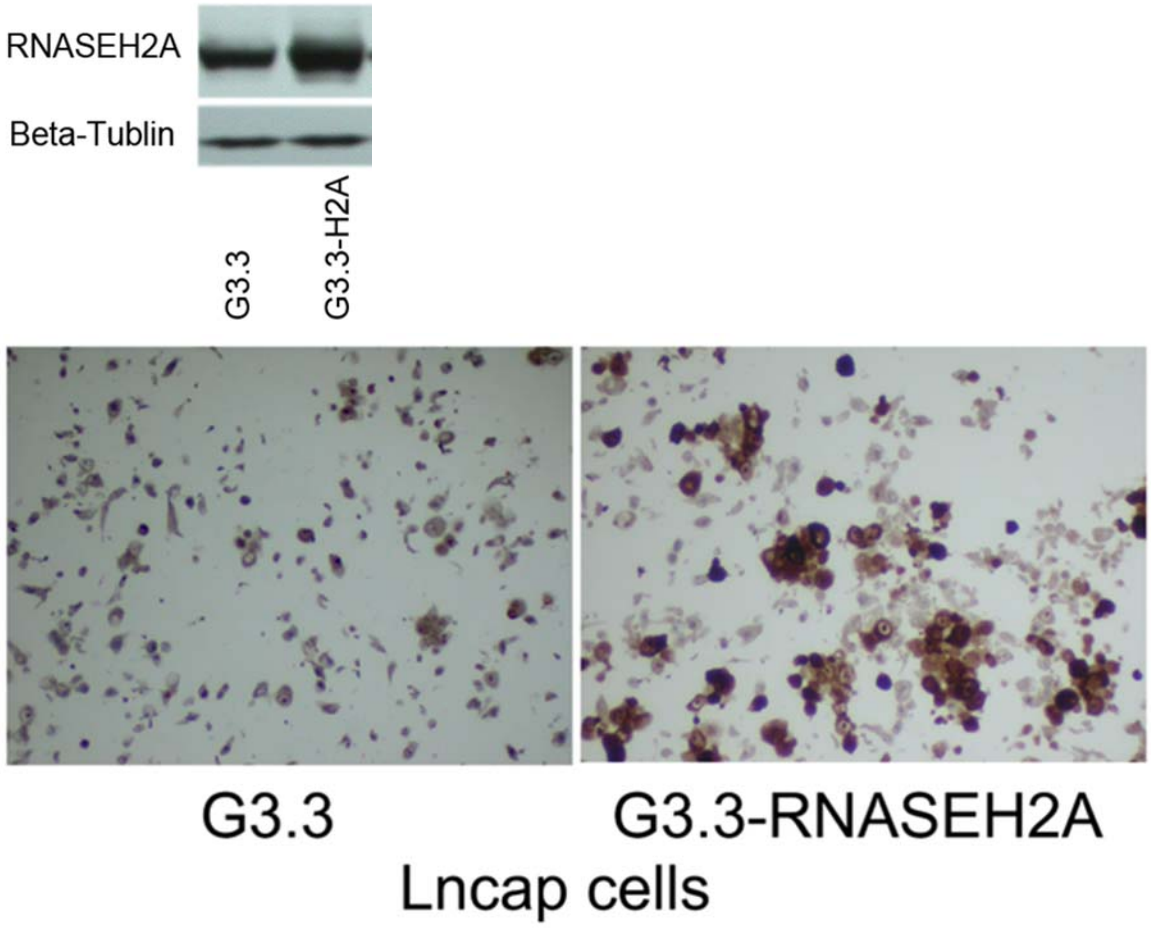


Figure 4 Western analysis (top panel) and immunohistochemistry (bottom images) of LnCap cells over expressing RNAH2A.

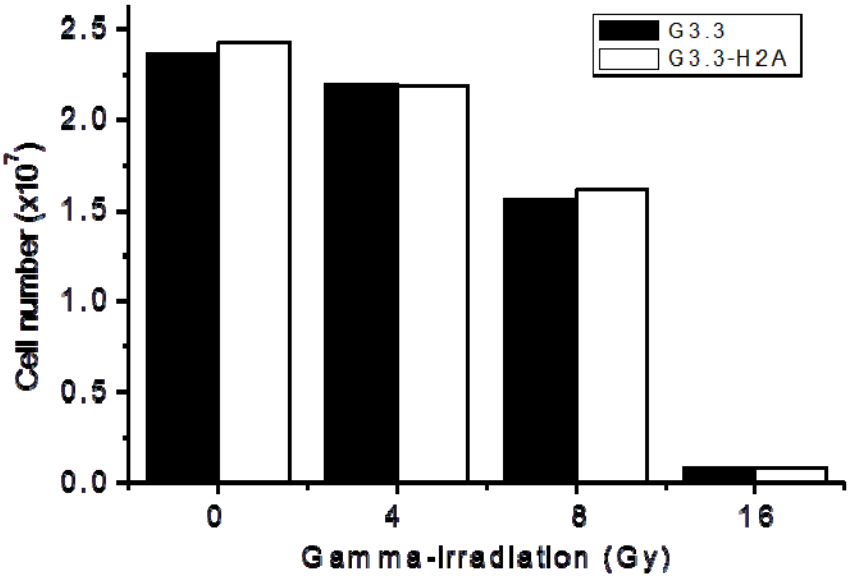


Figure 5. RNASH2a overexpression in LnCaP cells does not protect from ionizing radiation.

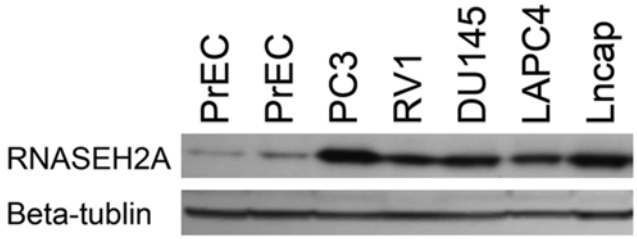


Figure 6. Western analysis of RNASH2a in various prostate epithelial cell lines.

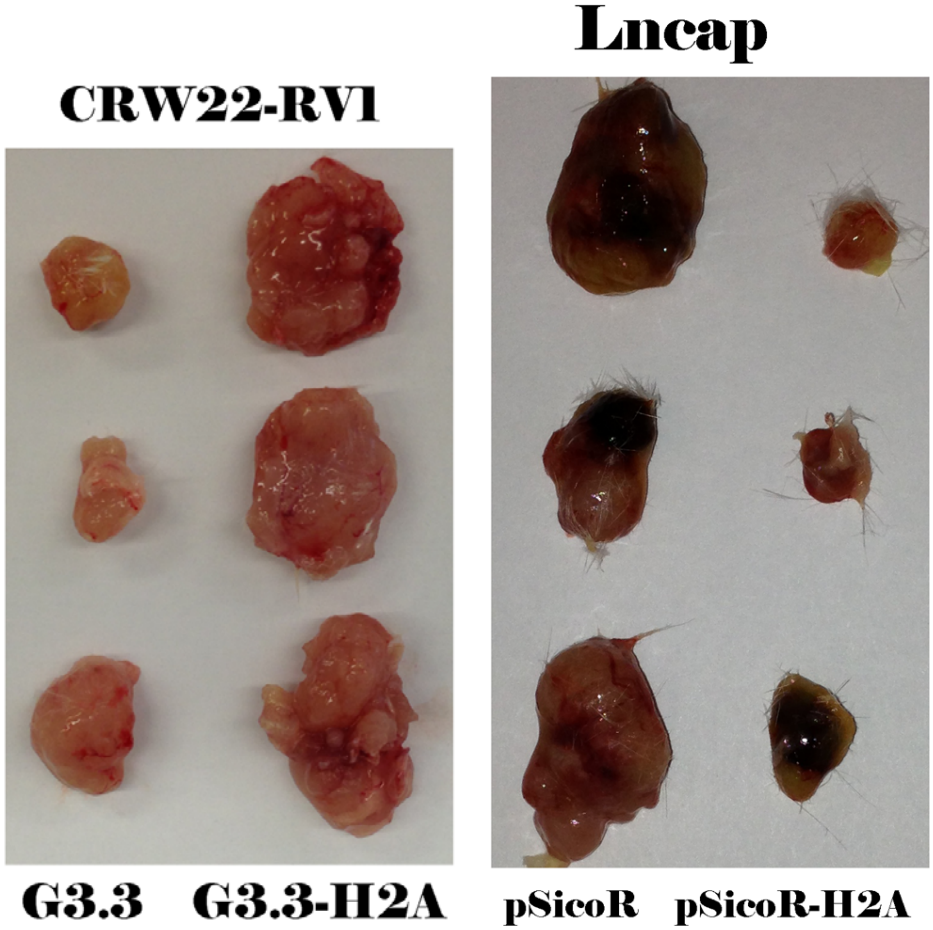


Figure 7. Xenograft from CWR22 cells over-expressing (Left panel) or LnCap cells under-expressing RNASH2A.